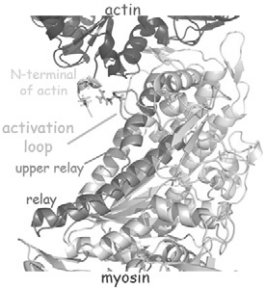
mechanism was still unrevealed. We demonstrate a new, conserved actin binding region, called activation loop. It is located at the relay region which swings the lever of myosin upon the powerstroke. We prove that activation loop interacts with the N-terminal segment of actin. We found that this interaction specifically activates myosin ATPase. Biochemical (steady state and transient kinetic fluorescent measurements) and *in vivo* experiments using transgenic *C. elegans* strains proved that activation loop is responsible for force production but not essential for motility *per se*. We conclude that actin binding to activation loop directly accelerates the lever movement. This process increases the ratio of working myosin heads and produce effective muscle contraction.



706-Pos Board B506

Interaction of a Novel Fluorescent Non-Nucleotide ATP Analogue with ATP-Driven Molecular Motors

Keiko Tanaka, Taro Kimura, Shinsaku Maruta.

Fluorescent nucleotide analogues are essential for analysis of nucleotide-binding proteins. Most of fluorescent-labeled ATP analogues are ribose-modified. However, they are known to be 2' and 3' isomers mixture. Often these isomers show different properties each other. To avoid isomers, we designed and synthesized non-nucleotide fluorescent ATP-analogue, *N*-methylanthraniloyl amino ethyl triphosphate (MANTTP) which similar structure to the non-nucleotide ATP analogue 2-[(4-azido-2-nitrophenyl) amino] ethyl triphosphate (NANTP). It is known that NANTP are good substrate for skeletal myosin and induces actin gliding in vitro motility assay. Excitation and emission maximums in the fluorescence spectrum MANTTP were 330nm and 430nm, respectively. MANTTP was hydrolyzed by conventional kinesin and skeletal myosin, and induced dissociation of acto-myosin. The MANTTPase of myosin and kinesin were significantly activated by actin and microtubule, respectively. The ADP form of MANTTP showed the formation of skeletal muscle myosin-MANTDP-BeFn complex which mimic the transient state in ATPase cycle and this complex detaches from actin filament. K_{SV} of MANTDP-S-1-phosphate analogue complex showed significantly smaller value than that of free MANTTP. The results suggested that the fluorophore moiety of MANTDP in the complex is buried deeply in the ATP binding site. The fluorescent intensity of MANTTP itself does not change on binding to myosin ATP binding site, however, MANTTP showed significant FRET between intrinsic tryptophan residue of skeletal muscle myosin and MANTTP. The binding of MANTTP to myosin can be observed as fluorescence increase on the stopped flow system. The second-order rate constant for MANTTP first binding to myosin is $0.15\times10^{-6}M^{-1}s^{-1}$. It was shown that the novel fluorescent ATP analogue is applicable to the kinetic studies on ATPases.

Electron and Proton Transfer

707-Pos Board B507

Proton Conduction via Water Wire in the Hv1 Proton Channel

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The voltage-gated proton (Hv1) channel (2006, Science 312: 589; 2006, Nature 440: 1213) is homologous to the voltage-sensing domain (VSD) of voltage-gated ion channels, but unlike ion channels, Hv1 lacks a central pore domain. In Hv1, which forms a dimer, but also functions as a monomer, the VSD serves dual functions: it gates the proton current and also acts as the proton conduction pathway (2008, Neuron 58: 546; 2008, PNAS 105: 9111). In order to understand the proton conduction mechanism in Hv1, we have performed all-atom simulations of Hv1 and its mutants in a lipid bilayer in excess water to an aggregate trajectory length that exceeds 1 μs. To generate our Hv1 structural model, we used the VSD structure of the Kv1.2 paddle-chimera channel (2007, Nature 450: 376) as a template. Because the Hv1 S4 helix contains only three of the four highly conserved arginines (R1-R4) that are known to confer voltage sensitivity in VSDs of Kv channels, we generated two initial model configurations; one where the Hv1 S4 arginines were aligned to R1-R3 of the Kv VSD structure, and a second one where they were aligned to R2-R4. In both models, we observe a water wire that extends through the membrane, and is single file over a stretch of 8Å. In contrast, in a control simulation of the Kv chimera VSD, no waters are observed in the corresponding region. A network of charged residues that includes the S4 arginines as well as several acidic residues coordinates the Hv1 water wire. The presence of multiple basic and acidic residues in the region central to the water wire may explain the robustness of proton conduction in the presence of a variety of mutations (2010, NSMB 17: 869).

708-Pos Board B508

Bovine Cytochrome Oxidase Structures Enable Molecular Oxygen Reduction without Formation of Active Oxygen Species, Providing a Proton Pumping Gate

Shinya Yoshikawa.

The O_2 reduction site of cytochrome c oxidase (CcO), comprising iron (Fe_{a3}) and copper (Cu_B) ions, is probed by X-ray structural analyses of CO, NO and CN^- derivatives to investigate the mechanism of the complete reduction of O_2. Formation of the $Fe_{a3}{}^{2+}$-CN^- derivative contributes to the trigonal planar coordination of $Cu_B{}^{1+}$ and displaces one of its three coordinated imidazole groups while a water molecule becomes hydrogen-bonded to both the CN^- ligand and the hydroxyl group of Tyr244. When O_2 is bound to $Fe_{a3}{}^{2+}$, it is negatively polarized (O_2^-), and expected to induce the same structural change induced by CN^-. This allows O_2^- to receive three electron equivalents non-sequentially from $Cu_B{}^{1+}$, $Fe_{a3}{}^{3+}$ and Tyr-OH, providing complete reduction of O_2 with minimization of production of active oxygen species.

The proton pumping pathway of bovine C*c*O comprises a hydrogen bond network and a water channel which extend to the positive and negative side surfaces, respectively. Protons transferred through the water channel are pumped through the hydrogen-bond network electrostatically with positive charge created at the Fe_a center by electron donation to the O_2 reduction site. Binding of CO or NO to $Fe_{a3}{}^{2+}$ induces significant narrowing of a section of the water channel near the hydrogen-bond network junction, which prevents access of water molecules to the network. In a similar manner, O_2 binding to $Fe_{a3}{}^{2+}$ is expected to prevent access of water molecules to the hydrogen-bond network. This blocks proton back-leak from the network and provides an efficient gate for proton pumping.

709-Pos Board B509

Preferred Pathway of Electron Transfer in the Dimeric Cytochrome b_6f Complex: Selective Reduction of One Monomer

S. Saif Hasan, Stanislav Zakharov, William A. Cramer.

The cytochrome b_6f and bc_1 (cyt *bc*) complexes are symmetric dimeric structures that enclose a central cavity that traps lipophilic quinone/ol from the membrane. The function of the dimer in electron transfer between the two hemes, b_p and b_n, is not known. Cross-over between the two hemes b_p was predicted[1] and recently observed.[2,3] although the branching ratio for cross-over is not known. Consideration of inter-heme distances in the photosynthetic bacterial bc_1 complex,[4] and in a range of cytochrome *bc* complexes[5] imply that the intra-monomer pathway (b_p-b_n) is significantly preferred over a pathway involving cross-over between the two hemes b_p. Split circular dichroism spectra in the Soret band of cytochrome *bc* complexes, (node of the CD spectra in the Soret band coincides with the absorbance maximum of hemes) imply that two *b*-hemes interact excitonically.[6] Consideration of inter-heme distances and angular orientations imply that exciton interaction in the Soret absorbance band detected by CD arises between reduced hemes b_p and b_n. Reduction of the b_6f complex by NADPH-ferredoxin in thylakoid membranes,[7] or for cyanobacterial b_6f complex isolated in detergent,[8] accomplishes the reduction of no more than half of the *b* heme in the complex. It is inferred that enzymatic reduction by FNR bound to the complex reduces hemes b_p and b_n in only one monomer of the dimer, perhaps because only one FNR can occupy the *n*-side (stromal) docking site of the dimer. It is inferred that inter-monomer b_p - b_p electron crossover is inefficient in this experiment (Support: NIH GM38323). *References*: [1]Soriano *et al*., 1999; [2]Swierczek *et al*. 2010; [3]Lanciano *et al*. 2010; [4]Shinkarev & Wraight 2007; [5]Cramer *et al.* 2010, in preparation; [6]Palmer & Degli-Esposti 1994; [7]Furbacher *et al*. 1989; [8]Yamashita *et al*. 2007.

710-Pos Board B510

Design of Transmembrane Electron Transport Chain within Amphiphilic Protein Maquettes

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Electron transport chains are fundamental to both photosynthesis and oxidative phosphorylation. Protein-based electron transport chains transfer electrons from high-energy donors to lower-energy acceptors and are commonly coupled to the translocation of protons across a membrane, producing a transmembrane electrochemical potential gradient. Electron transfer rates within these chains are governed primarily by the distance between redox centers and by the driving force that originates from the redox mid-point potentials or coupled catalytic reactions. The complexity of natural redox protein structures contrasts the relatively simple rules of cofactor placement that, in principle, govern the electron transfer behavior.